

IN THE CLAIMS:

1. (Previously amended) A method of classifying and counting leukocytic cells and erythroid cells in a bone marrow fluid comprising leukocytic cells and erythroid cells and lipid particles comprising the steps of:

(1) (a) mixing a sample of the bone marrow fluid with an erythrocyte lysing agent to lyse erythrocytes in the sample, thereby rendering leukocytic cells, erythroid cells and lipid particles in the sample suitable for staining, and

(b) staining the sample with a fluorescent dye for producing a difference in intensity of fluorescence among the leukocytic cells, the erythroid cells, and the lipid particles;

(2) introducing the resulting sample to a flow cytometer to detect at least one kind of scattered light and at least one kind of fluorescence;

(3) classifying the lipid particles, the leukocytic cells and the erythroid cells by the difference in the intensities of their fluorescence and their scattered light; and

(4) obtaining a count of the leukocytic cells and erythroid cells in the step of (3).

2. (Previously amended) The method according to claim 1, further comprising the steps of:

classifying erythroid cells into at least two erythroid cell groups according to maturity of each of the erythroid cells, and obtaining a count of cells in each of the erythroid cell groups by the difference in the intensities of the fluorescence and the scattered light from the at least two erythroid cell groups; and

calculating the ratio of the classified cells in each of the erythroid cell groups to the total erythroid cell count.

3. (Previously amended) The method according to claim 1, further comprising the steps of:

classifying lymphocytes and monocytes included in the leukocytic cells and obtaining a lymphocyte count and a monocyte count; and

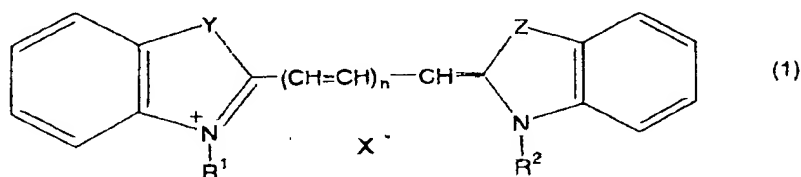
calculating a myeloid cell count by deducting the obtained lymphocyte count and the obtained monocyte count from the leukocytic cell count; and

calculating the ratio of the erythroid cells to myeloid cells from the obtained myeloid cell count and erythroid cell count.

4. (Original) The method according to claim 1, wherein the erythrocyte lysing agent is an aqueous solution having an osmotic pressure of 100 mOsm/kg or less and a pH of 2.0 to 5.0.

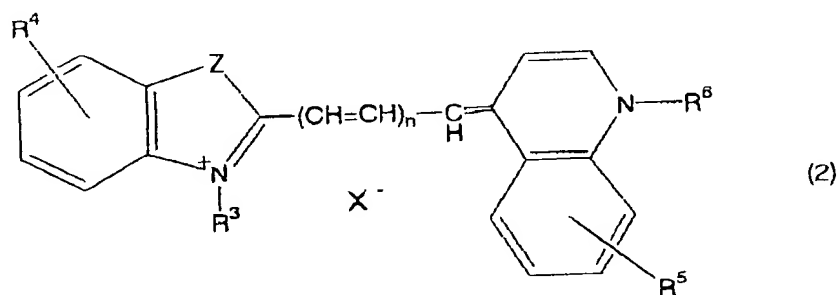
5. (Original) The method according to claim 1, wherein the fluorescent dye comprises one or more dyes selected from the group consisting of:

- compounds of formula (1)



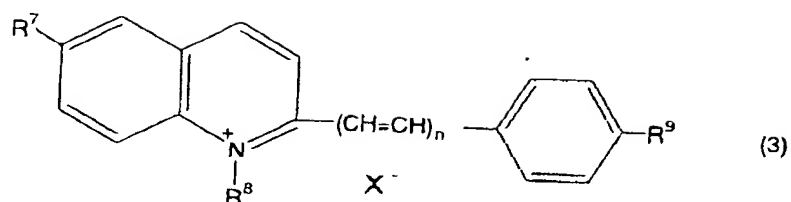
wherein R¹ and R² are, the same or different, a hydrogen atom, or an alkyl or alkenyl group optionally substituted by a hydroxyl group; Y and X are, the same or different, a hetero atom or a carbon atom substituted by a lower alkyl group; n is 0, 1 or 2; and x⁻ is an anion,

- compounds of formula (2)



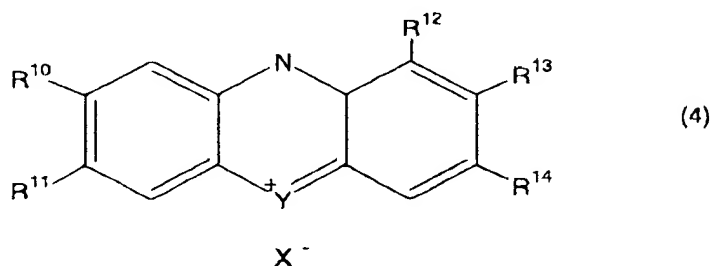
wherein R³ is a hydrogen atom or an alkyl group; R⁴ and R⁵ are, the same or different, hydrogen atom, a lower alkyl group or a lower alkoxy group; R⁶ is a hydrogen atom, an acyl group or an alkyl group; Z is a hereto atom or a carbon atom substituted by a lower alkyl group; n is 0, 1 or 2; and x⁻ is an anion,

-compounds of formula (3)



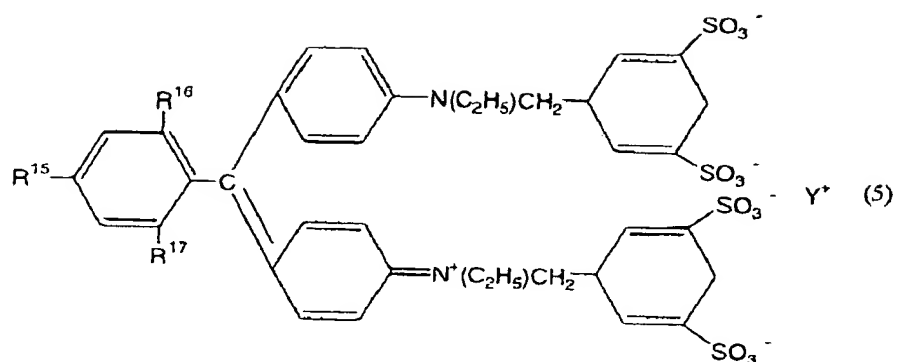
wherein R^7 is a hydrogen atom or a dimethylamino group; R^8 is an alkyl group; R^9 is a hydrogen group or a dimethylamino group; n is 1 or 2; and x^- is an anion,

-compounds of formula (4)



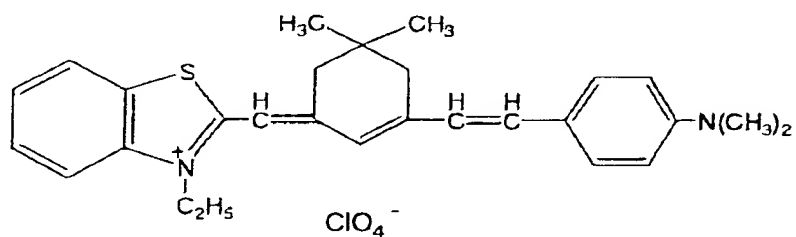
wherein R^{10} is a hydrogen atom or an alkyl group; R^{11} is a dimethylamino group; R^{12} is a hydrogen atom or an amino group; R^{13} is a hydrogen atom, an alkyl group or an amino group; R^{14} is a hydrogen atom or a dimethylamino group; X^- is an anion; and Y is a hetero atom,

-compounds of formula (5)

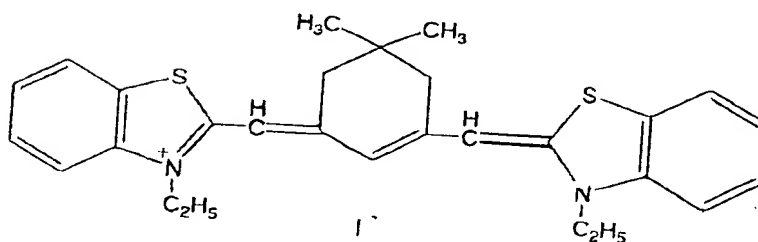


wherein R^{15} is a hydrogen atom or a hydroxyl group; R^{16} is a hydrogen atom or a sulfonic group; R^{17} is a hydrogen atom or a sulfonic group; and Y is a cation,

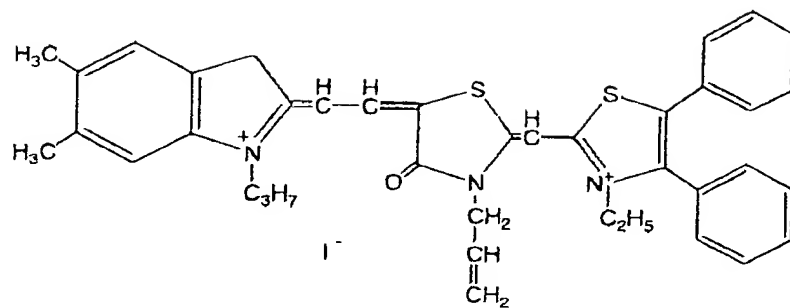
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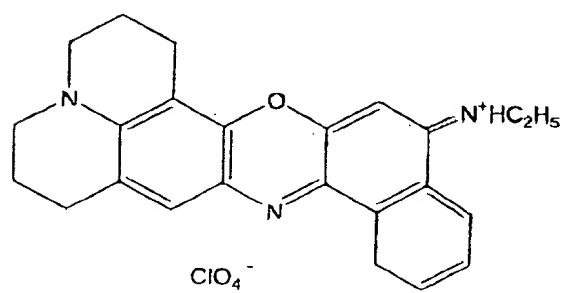
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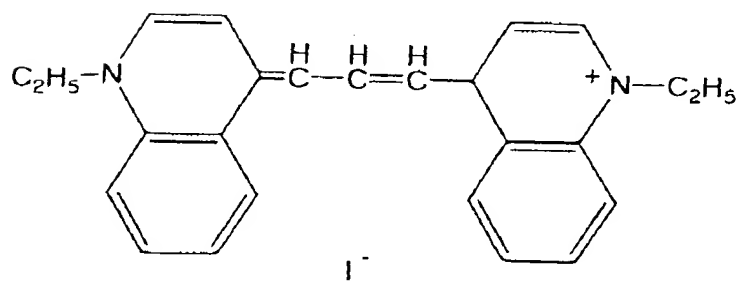
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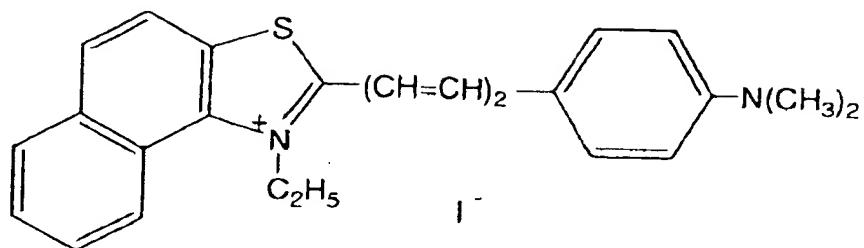
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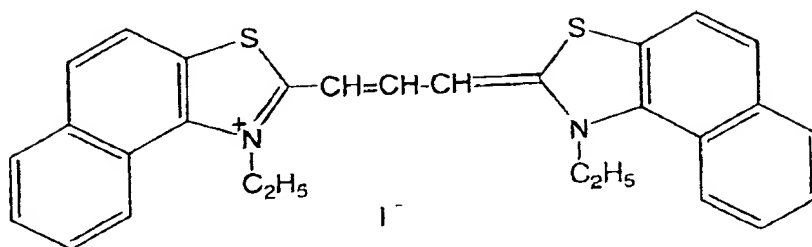
-Cryptocyanine:



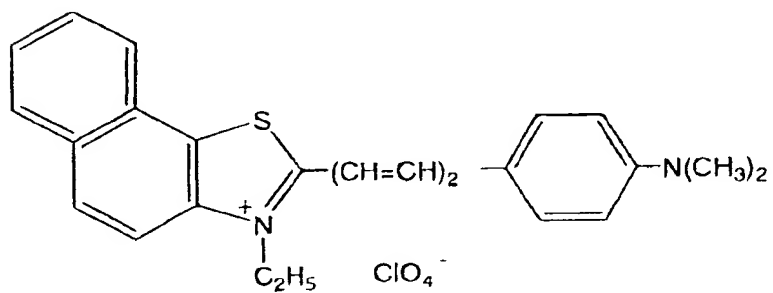
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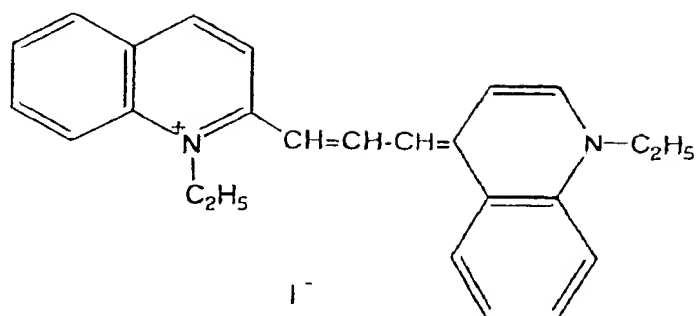
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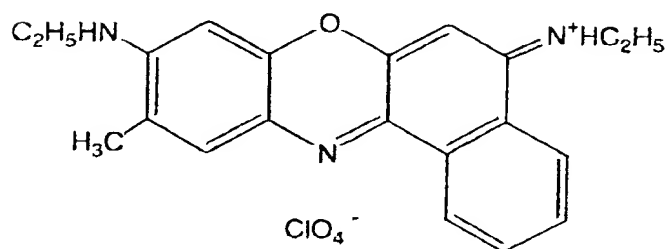
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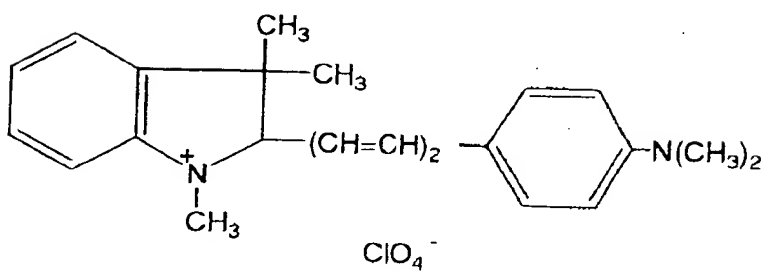
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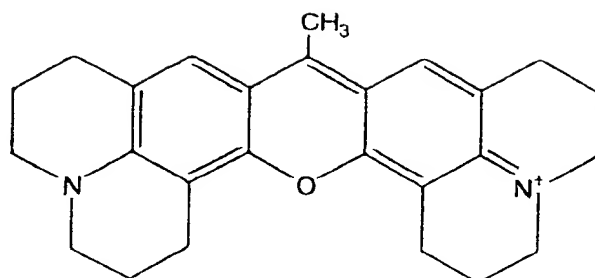
-Oxazine 720:



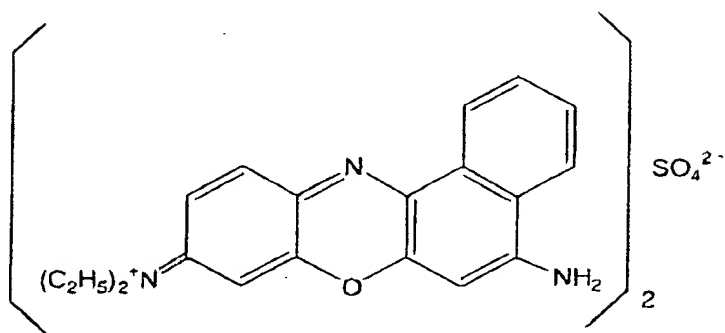
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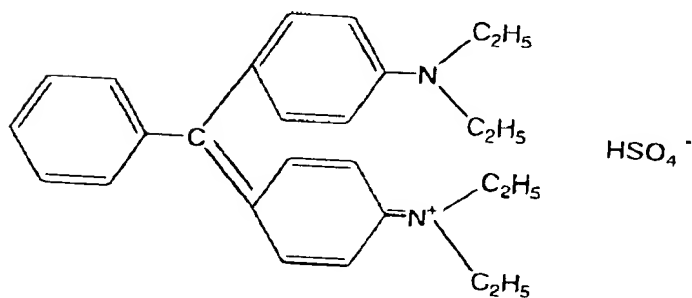
-LD700:



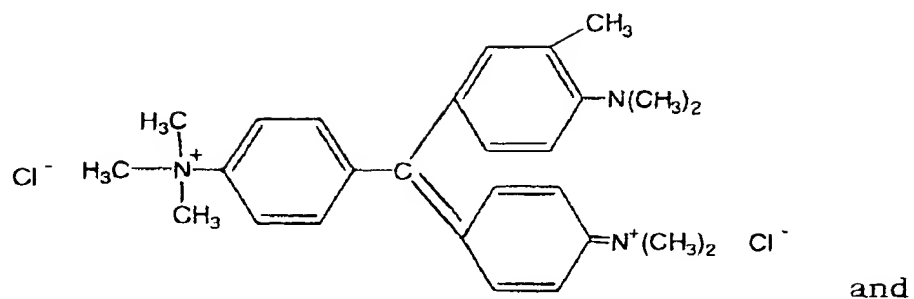
-Nile Blue A:



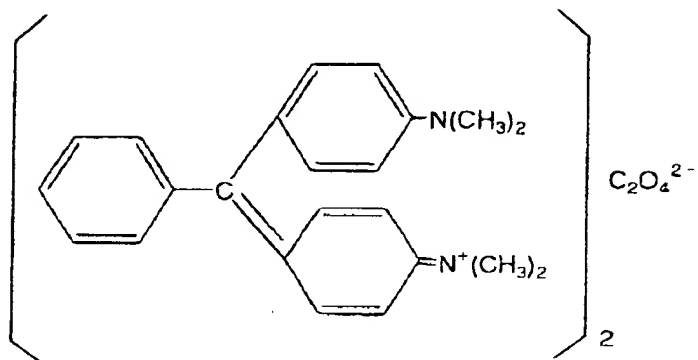
-Brilliant Green:



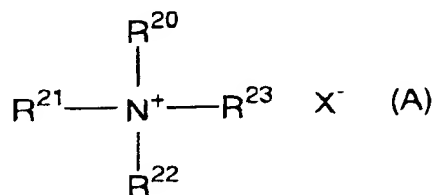
-Iodide green:



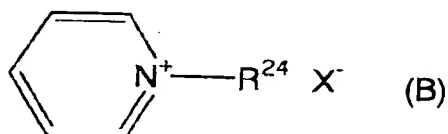
-Malachite green:



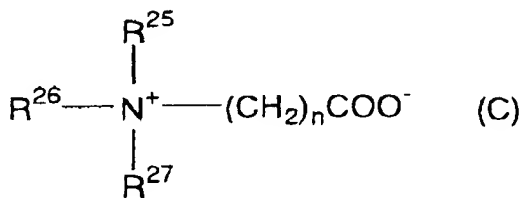
6. (Original) A method according to claim 1, wherein the erythrocyte lysing agent contains a surfactant, the surfactant comprises one or more surfactants selected from the group consisting of
-compounds of formula (A)



wherein R^{10} , R^{21} and R^{22} are, the same or different, an hydrogen atom, a C_{1-8} alkyl group or a C_{6-8} aralkyl group; R^{23} is a C_{8-18} alkyl group, a C_{8-18} alkenyl group or a C_{6-18} alkenyl group or a C_{6-18} aralkyl group; and x^- is an anion,
 -compounds of formula (B)



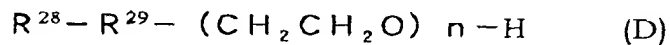
wherein R^{24} is a C_{8-18} alkyl group; and X^- is an anion,
 -compounds of formula (c)



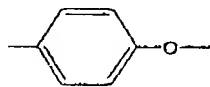
wherein R^{25} and R^{26} are, the same or different, a hydrogen atom, a C_{1-8} alkyl group, or a C_{6-8} aralkyl group; R^{27} is a C_{8-18} alkyl group, a C_{8-18} alkenyl group or a C_{6-18} aralkyl group; and n is 1 or 2,
 -compounds of formula (D)

wherein R^{25} and R^{26} are, the same or different, a hydrogen atom, a C_{1-8} alkyl group, or a C_{6-8} aralkyl group; R^{27} is a C_{8-18} alkyl group, a C_{8-18} alkenyl group or a C_{6-18} aralkyl group; and n is 1 or 2,

- compounds of formula (D)

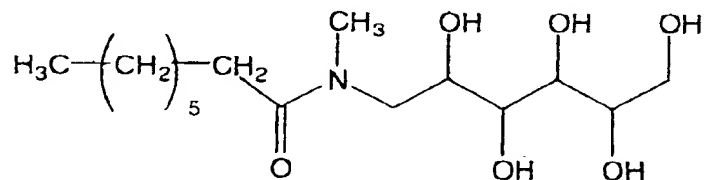


wherein R^{28} is a C_{9-25} alkyl group, a C_{9-25} alkenyl group or a C_{9-25} alkynyl group; R^{29} is

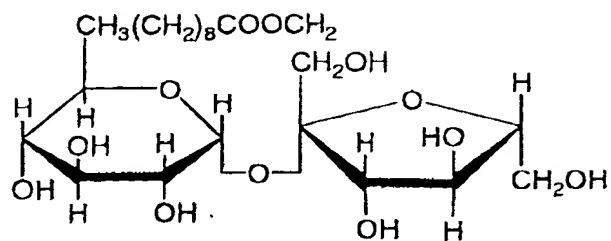


or $-COO-$; and n is an integer of 10 to 40,

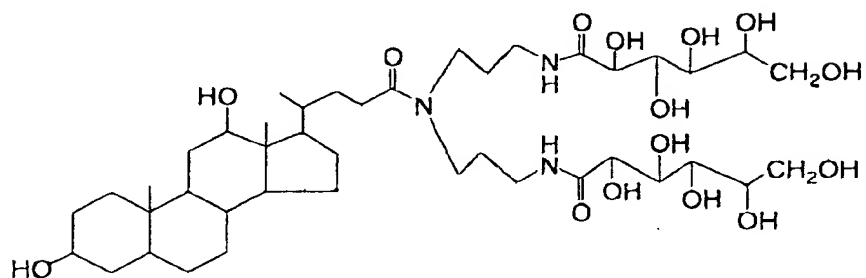
-MEGA-8:



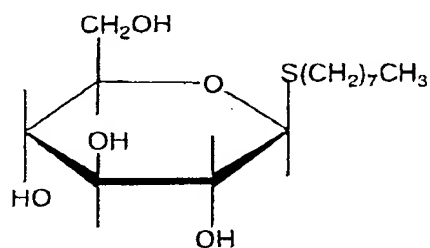
-sucrose monocaproate:



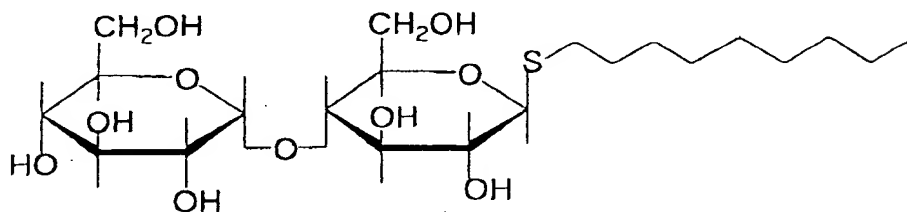
-Deoxy-BIGCHAP:



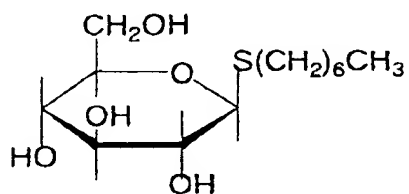
-n-octyl- β -D-thioglucoside:



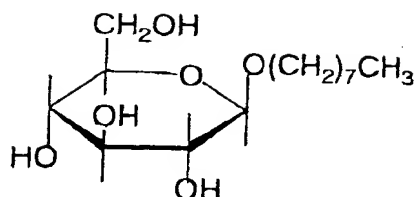
-n-nonyl- β -D-thiomaltoside:



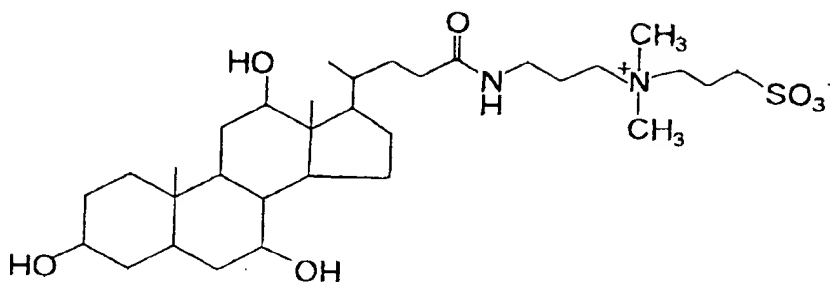
-n-heptyl- β -D-thioglucoside:



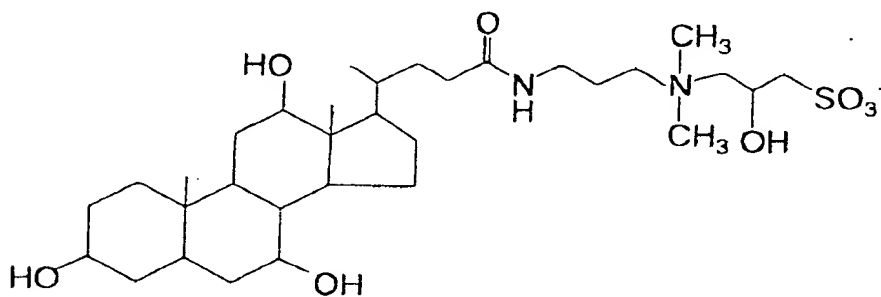
-n-octyl-D-oxyglucoside:



-CHAPS:



-CHAPSO:



7. (Original) The method according to claim 6, wherein the concentration of the surfactant is 10 to 10000 mg/L.

8. (Original) The method according to claim 1, wherein the detected scattered light is one or more kinds of scattered light selected from the group consisting of forward low-angle scattered light, forward high-angle scattered light and side scattered light.

9. (Previously presented) The method according to claim 1, further comprising the step of:

calculating the ratio of the total count of leukocytic cells and erythroid cells to the count of erythroid or leukocytic cells.

10. (Previously presented) The method according to claim 1, further comprising the step of:
calculating the ratio of the obtained leukocytic cell count to the obtained erythroid cell count.

11. (New) A method of classifying and counting leukocytic cells and erythroid cells in a bone marrow fluid comprising leukocytic cells and erythroid cells and lipid particles comprising the steps of:

(1) (a) mixing a sample of the bone marrow fluid with an erythrocyte lysing agent to lyse erythrocytes in the sample, thereby rendering leukocytic cells, erythroid cells and lipid particles in the sample suitable for staining, and

(b) staining the sample with a fluorescent dye for producing a difference in intensity of fluorescence among the leukocytic cells, the erythroid cells, and the lipid particles;

(2) introducing the resulting sample to a flow cytometer to detect side scattered light and at least one kind of fluorescence;

(3) classifying the lipid particles, the leukocytic cells and the erythroid cells by the difference in the intensities of their fluorescence and their scattered light; and

(4) obtaining a count of the leukocytic cells and erythroid cells in the step of (3).